AP Enzyme Kinetics Lab

In this lab you will first record a baseline value for the rate at which the enzyme peroxidase converts hydrogen peroxide into water & oxygen. This baseline will establish the rate of the enzyme under standard conditions. Each group/member will then be assigned a variable to test the effects of on the enzyme.
You will use a tool called a spectrophotometer which measures color change. The reaction between peroxide and peroxidase will be easily visible by adding an indicator called guaiacol which turns from clear to brown during the reaction. This change in color will be measured by the spectrophotometer and provide a value called absorbance. Absorbance refers to how much light is absorbed by a solution in the spectrophotometer so as the solution becomes darker, the absorbance value will increase.

The variables you will test are as follows

2x enzyme	1/2x enzyme	2x substrate	1/2x substrate
1/4 x substrate	Higher pH (pH 8)	Lower pH (4)	NaCl solution

Blank Procedures
1. Add 2mL of the "S" tube (see below) to a cuvette.
2. Place into spectrophotometer.

Baseline Procedures
1. In a test tube "S" add 7mL H_2O, 1mL H_2O_2 and 0.5mL guaiacol.
2. In a second test tube "E" add 7mL H_2O and 1.5mL peroxidase enzyme.
3. Add 1mL of tube "S" contents into a cuvette then carefully place the cuvette into the Spec.
4. CAREFULLY add 1 mL of tube "E" contents in the cuvette and hit the start button on the Spec.

Table 1: Baseline Absorbance

Time (s)	Absorbance (nm)
0	0.017
30	0.239
60	0.496
90	0.694
120	0.840
150	0.946
180	1.020
210	1.080
240	1.122
270	1.165
300	1.203

Experimental Procedures (Be sure to calibrate (blank) before each trial)

Use table 2 below to determine the proper amount of solutions to add to your tubes.

Table 2: Different variable volumes for reaction tubes

Tube 1	2x enz	1/2 enz	2x sub	1/2 sub	1/4 sub	Low pH	High pH	NaCl
Peroxide	1mL	1mL	2mL	0.5ml	0.25mL	1mL	1mL	1mL
Water	7mL	7mL	6mL	7.5mL	7.75mL	2mL	2mL	2mL
Guaiacol	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL
8 buffer	X	X	X	X	X	X	5mL	X
4 buffer	X	X	X	X	X	5mL	X	X
NaCl	X	X	X	X	X	X	X	5mL
Tube 2	2x enz	1/2 enz	2x sub	1/2 sub	1/4 sub	Low pH	High pH	NaCl
Enzyme	3mL	0.75mL	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5mL
Water	5.5mL	7.75mL	7mL	7mL	7mL	7mL	7mL	7mL

2x enzyme

Time (s)	Absorbance (nm)
0	0.027
30	0.720
60	1 084
90	1.259
120	1.371
150	1.454
180	1 512
210	1.555
240	1.598
270	1.625
300	1 658

1/2 enzyme

Time (s)	Absorbance (nm)
0	-0.292
30	-0.187
60	-0. 058
90	0.041
120	0 118
150	0.176
180	0.219
210	0.255
240	0.286
270	0 314
300	0 339

2x substrate

Time (s)	Absorbance (nm)
0	-0.445
30	-0.128
60	0.068
90	0.179
120	0.246
150	0.301
180	0.345
210	0.379
240	0.417
270	0.446
300	0.488

1/2 substrate

Time (s)	Absorbance (nm)
0	0.018
30	0.184
60	0.333
90	0.437
120	0.505
150	0.561
180	0.606
210	0.644
240	0.683
270	0 718
300	0.750

1/4 substrate

Time (s)	Absorbance (nm)
0	-0.043
30	0.073
60	0.216
90	0.325
120	0 405
150	0.479
180	0.542
210	0.599
240	0.646
270	0 687
300	0.725

pH 4

Time (s)	Absorbance (nm)
0	-0.017
30	0.188
60	0.404
90	0 577
120	0.726
150	0.843
180	0.941
210	1.028
240	1.105
270	1161
300	1.209

pH 8

Time (s)	Absorbance (nm)
0	0.019
30	0.081
60	0 165
90	0.241
120	0.314
150	0.374
180	0.438
210	0.500
240	0.554
270	0.606
300	0.647

NaCl

Time (s)	Absorbance (nm)
0	0.015
30	0.302
60	0.527
90	0.709
120	0.862
150	0.990
180	1.092
210	1.198
240	1.276
270	1.352
300	1428

You will generate 7 total graphs:

- Baseline & 2 other enzyme concentrations as a function of time (3 lines)
- Enzyme rate as a function of enzyme concentration (1 line)
- Baseline & 3 other substrate concentrations as a function of time (4 lines)
- Enzyme rate as a function of substrate concentration
- Baseline & 2 other pH levels as a function of time (3 lines)
- Enzyme rate as a function of pH (3 lines)
- Baseline & NaCl as a function of time (2 lines)

Analysis

1. **Draw conclusions** about the variables - varying enzyme concentration, varying substrate concentration & varying pH level, adding an ionic compound (NaCl)
Explain why the changes occurred on a **molecular level** using the terms active site, substrate, denaturation and Vmax where appropriate.

2. **Predict** the effects of varying temperature on enzyme activity and explain in terms of molecular changes to the enzyme.

One question not addressed is how inhibitors affect enzymes. The graph below shows typical results for comparing a baseline with competitive and non-competitive inhibitors. Some terms are defined below.

Vmax = maximum velocity of the enzyme; used to determine *maximum* rate of the enzyme as a function of increasing substrate concentration.

1/2 Vmax = Half the value of Vmax; used as a more accurate measure of enzyme's *steadiest rate* as a function of increasing substrate concentration.

Km = The **substrate concentration** at the **1/2 Vmax value** (Used to determine how *efficiently* the enzyme is converting substrate to product.

[S] = Concentration of the substrate; used to determine how the enzyme functions from low to high levels of substrate.

3. **Refine** our lab to include a means of determining whether a substance would be a competitive inhibitor or a non-competitive inhibitor. Be sure to explain required lab procedures and analysis procedures in order to determine this (hint – use the graph below for analysis!) Also, **explain** on a molecular level why competitive inhibitors have different Km and same Vmax as a baseline BUT non-competitive inhibitors have a different Vmax and same Km as a baseline.